North Carolina
Lab-Oratory
N.C. Department of Health and Human Services / State Laboratory of Public Health

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From the Director's Chair

Organizational Changes

Leslie A. Wolf, PhD, HCLD (ABB)
Laboratory Director

Since the last issue of *Lab-Oratory* was printed, State Health Director Dr. Leah Devlin has announced the following organizational changes within the Division of Public Health: Dr. Steve Cline, formerly Epidemiology Section Chief, has been named Deputy Health Director; Dr. Jeff Engel, State Epidemiologist, now has primary responsibility for the General Communicable Disease Branch (GCDC), Occupational and Environmental Epidemiology Branch, and HIV/STD Prevention and Care Branch. Dr. Lou Turner has been assigned primary responsibility at the Section level in overseeing the Office of Public Health Preparedness and Response, the Office of the Chief Medical Examiner, and the State Laboratory of Public Health, and I am to serve as the Director of the State Laboratory of Public Health.

New Laboratory Facility

Our mission here at NCSLPH is to provide certain medical and environmental laboratory services (testing, consultation and training) to public and private health provider organizations responsible for the promotion, protection and assurance of the health of North Carolina citizens. Since September 11, 2001 and the anthrax crisis that began in October 2001, SLPH has been expected to play a key role in preparedness

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MISSION statement

The State Laboratory of Public Health provides certain medical and environmental laboratory services (testing, consultation and training) to public and private health provider organizations responsible for the promotion, protection and assurance of the health of North Carolina citizens.

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activities. In order to provide the highest quality environmental and clinical tests, the Lab uses state-of-the-art, sophisticated instrumentation. Unfortunately, the Bath Building does not provide sufficient space, adequate air handling or "clean" power required for optimal operation of this instrumentation. Thanks to the leadership of a number of people, in particular NC DHHS Secretary Carmen Hooker Odom, Dr. Leah Devlin and Dr. Lou Turner, the dream of a new facility is becoming a reality, with the General Assembly approving $101 million for this purpose during the short session. Planning has already started and we are thrilled with this development!

CIPHER: Pandemic Influenza Exercise

During the latter part of May 2006, a large state-wide exercise was conducted to test the N.C. Pandemic Influenza Plan, specifically the ability of different agencies to collaborate, cooperate, and communicate. The theme of this exercise was the elevation by the World Health Organization (WHO) of influenza pandemic levels to 5 and 6 and subsequently, the arrival of the H5N1 strain of avian influenza in North Carolina. Each agency at the local, state and federal level was expected to respond appropriately to the N.C. Plan. The State Emergency Operations Center was opened, as was the Public Health Command Center (PHCC). The Incident Command System (ICS) was utilized during the exercise to effectively manage such a large event, and SLPH Assistant Director of Operations, Mike Kaufman, served as Incident Commander at the PHCC. While there were many lessons learned from the Collaborative and Integrated Public Health, Hospital, and Emergency Response (CIPHER), exercise participants found that for many of the situations that occurred, the N.C. Pandemic Influenza Plan was an excellent guide. Please see the website listed below for more information.

Pandemic Influenza Activities

NCSLPH worked closely with General Communicable Disease Control (GCDC) Branch staff to develop the N.C. Pandemic Influenza Plan, available on the web at *http://www.epi.state.nc.us/epi/gcdc/pandemic.html*. NCLSPH participated in the May 2006 statewide CIPHER Pandemic Influenza Exercise in which the laboratory portion of the N.C. Pandemic Influenza plan was tested. The laboratory appendices H-1 and H-2 of the N.C. plan includes the algorithm NCSLPH will follow based on information provided by submitters or GCDC epidemiologists about patient signs and symptoms, travel history, animal exposure or exposure to another patient with influenza-like symptoms, and other laboratory results. In the event that the patient is not recognized as being at risk of having a novel influenza virus, and a sample is submitted to NCSLPH, safety precautions have been considered. NCSLPH protocols in Viral Culture/Rabies laboratory includes special handling steps for routine isolates that do not exhibit expected subtyping results because these isolates may represent pandemic influenza strains.

Molecular subtyping will be performed to characterize influenza isolates. The NCSLPH implemented two molecular methodologies: conventional and real-time RT-PCR to characterize influenza isolates. Conventional RT-PCR consists of single reactions containing type A (matrix protein gene) and type B (nonstructural gene) specific primers, subtype H1 and H3 specific primers, and/or subtype N1 and N2 specific primers, extracted influenza viral RNA, reverse-transcriptase enzyme, polymerase enzyme, RNase inhibitor enzyme, and buffer. RT-PCR is performed to synthesize complementary DNA strand (cDNA). This step is followed by PCR which amplifies the specific genomic target. The resulting amplified DNA products are analyzed by gel electrophoresis. The expected product sizes are as follows: Type A- 311bp, Type B-108bp, H1-164bp, H3-232bp, N1-106bp, and N2-173bp. Conventional RT-PCR analysis results can be generated within two to three days. However, real-time RT-PCR analysis can be generated within eight hours of specimen receipt. Influenza typing primer/probe sets for real-time RT-PCR are designed for universal detection of type A and type B influenza viruses. Influenza A subtyping primer/probe sets are designed to specifically detect modern human influenza viruses H1 (H1), human H3 (H3), and Asian avian influenza (AI) virus H5 (H5). The NCSLPH utilizes two real-time thermocycler systems [BioRad iCycler iQTM and Roche Lightcycler (LRN H5 protocol)], for isolate characterization.

Avian Influenza Activities

Testing of the novel H5 avian influenza (AI) strain involves several Units of the laboratory, including Administration, Virology/Serology, and Bioterrorism/Emerging Pathogens. An AI Team has been assembled and meets on a regular basis to ensure the NCSLPH is operating in a unified manner. The Laboratory Response Network (LRN) released a real-time molecular method for H5 influenza in 2005-2006. The Bioterrorism and Emerging Pathogens

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Unit has primary responsibility for utilizing this assay upon notification by GCDC Epidemiologists that a patient meets the criteria for testing. The three N.C. Regional Response Laboratories (managed by the Bioterrorism & Emerging Pathogens Unit) are projected to have the LRN assay on-line by late September/early October.

Case history (travel history and exposure to suspected or confirmed AI infected birds or humans) should be provided to GCDC and NCSLPH. If there is a suspected case of novel or AI, the specimen is not inoculated into cell culture. The primary sample is taken directly to the BSL-3 area and nucleic acid is extracted for use in real-time RT-PCR to rule out AI. If the sample is negative for H5 using real-time RT-PCR, it is typed for influenza A or B and sub-typed for H1/H3. Since 2005, the NCSLPH has ruled out H5 for seven suspected AIV patients. In four of the seven specimens, influenza A/H3 RNA was detected by RT-PCR.

Leslie A. Wolf, PhD, HCLD (ABB)
Laboratory Director

The Significance of Hemoglobin A_2' in the Diagnosis of Beta Thalassemia

The Hemoglobinopathy Laboratory at NCSLPH detects abnormal hemoglobins in infants and adults. Although primary emphasis lies in detection of sickle cell diseases, various other abnormal hemoglobins are also identified. Among them is Hgb A_2' (A2 Prime). Most health care providers are familiar with the role of Hgb A_2 in the diagnosis of beta thalassemia, an inherited condition that affects the production of normal hemoglobin. An elevated Hgb A_2 is a defining characteristic of this disorder, along with a microcytic, hypochromic blood picture. When beta thalassemia is suspected, it is imperative to correlate an accurate percentage of Hgb A_2 with other clinical features.

Hgb A_2 is made up of two alpha and two delta globin chains and normally comprises approximately 3% of total adult hemoglobin. Hgb A_2' is formed when glycine is substituted by arginine at the 16th position of the delta globin chain. This variant is sometimes called Hgb B_2 and occurs in 1-2% of African Americans, making it the most common delta chain variant. Whether occurring in the heterozygous or homozygous state, the only significance of Hgb A_2' is its contribution to the total percentage of Hgb A2. In laboratory testing using Isoelectric Focusing (IEF) and High Performance Liquid Chromatography (HPLC), Hgb A_2' migrates separately from Hgb A_2. If the percentage of the A_2' band or peak is not added to the Hgb A_2 level, there will be an underestimation of the

Hb A_2' or Hb B_2	**õ16 (A13) Gly ⟶ Arg**
CONTACT	External
ELECTROPHORESIS	Hb X moves slower than Hb A_2 at alkaline pH
IEF	Same
CHROMATOGRAPHY	Hb X can be separated from Hb A and Hb A_2 by cation and anion exchange chromatography
STRUCTURE ANALYSIS	Tryptic digestion of õ chain: separation of peptides by fingerprinting; cation exchange chromatography or reversed phase HPLC; amino acid analysis
DNA ANALYSIS	A GGC-CGC mutation at codon 16
NOTES	HbA_2' is the most common õ chain variant; it is found mainly in Black families; it is observed in heterozygotes, homozygotes, in combination with Hb S, Hb C, ß-thal (in *cis* and *trans*)

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Hemoglobin cont. from page 3

total Hgb A_2 quantitative measurement. In cases where the physician is suspecting thalassemia, this becomes a crucial factor.

Results of recent proficiency testing surveys by the College of American Pathologists indicate that IEF and HPLC methodologies are much more likely to detect Hgb A_2' than is routine electrophoresis. The Hemoglobinopathy Laboratory at NCSLPH uses both methodologies for screening and confirming abnormal results and began reporting the presence of Hgb A_2' in 2003. The variant may first be detected when a blood spot is submitted for hemoglobin screening. The result will report as "A + Variant," and EDTA blood is requested for follow-up testing to confirm and identify the variant and to provide quantitations. If the variant confirms as Hgb A_2', the whole blood report will contain a comment briefly describing the identity of the variant. Quantitations will be given for hemoglobins A_2, A_2', and F. To further explain the significance of this variant, an A_2' information sheet (see attachment 1) will be attached to the report. When reviewing the total Hgb A_2 level, the physician should also take into account any other hematological abnormalities before making a diagnosis of thalassemia.

Hb A_2', whether heterozygous or homozygous, is clinically and hematologically silent. Its sole significance is that it may cause an underestimation of Hb A_2 levels in the work-up of thalassemia. An accurate Hb A_2 level for this purpose represents the sum of the Hb A_2 and Hb A_2' bands or peaks on alkaline electrophoresis, IEF, or HPLC. Thus, failure to recognize the presence of Hb A_2' will result in underestimation of the total Hb A_2 (Hb A_2 plus Hb A_2') by half. Note that Hb A_2' coelutes with Hb A_2 by microcolumn chromatography, and thus total Hb A_2 measured by this methodology would be expected to be accurate.

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Clinical Laboratory Day Provided a Forum of Learning for Multiple Health Professional Disciplines

The Diabetes Challenge: Diagnosis, Education, and Management was the focus for the 2nd annual North Carolina Clinical Laboratory Day on Aug. 4. Realizing that diabetes is causing growing healthcare concerns, Laboratory Improvement—the training unit within the North Carolina State Laboratory of Public Health (NCSLPH)—developed the program in partnership with the North Carolina Diabetes Prevention and Control Branch (NCDPCB). Conference cosponsors also included Texas Health Foundation and the North Carolina Public Health Nursing Continuing Education Advisory Committee. As they did for last year's inaugural event, the conference organizers obtained quality speakers and kept

North Carolina Area Health Education Centers display promotes their mission "to meet the state and health work force needs by providing educational programs in partnership with academic institutions, health care agencies and other organizations committed to improving the health of the people of North Carolina".

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the registration fee low ($30) by recruiting cosponsors and exhibitors. More than 150 people, including nurses, clinical laboratory scientists, medical office assistants, and diabetes educators, attended the one-day conference, which provided continuing education credits (6.0 contact hours).

Marcus Plescia, MD, MPH, Chief of the North Carolina Division of Public Health's Chronic Disease and Injury Section, gave the opening welcome for the event at Wake Technical Community College in Raleigh. He lauded the collaborative effort of the NCSLPH and the NCDPCB in providing this educational opportunity for health professionals. Director of the NCSLPH, Leslie Wolf, PhD, also expressed a warm welcome as she thanked the sponsors and exhibitors for their generous support. She acknowledged HemoCue, Inc. for their gold-level sponsorship, which provided funding to cover general costs of the program.

Dr. Wolf introduced the first speaker, Beverly Robertson, BS MT(ASCP), MPH. The Point-of-Care Testing Coordinator for the University of North Carolina Health Care System, Ms. Robertson mixed humor with her presentation in *"How Sweet It Is," The Role of the Lab in Glucose Monitoring*. She addressed laboratory tests for the detection and management of diabetes, including glucose, A1c hemoglobin, microalbumin, and cholesterol. She discussed the various test methods employed by point-of-care analyzers, laboratory glucose assays and self-monitoring glucose devices used by people with diabetes, which all use one of the following test methods: glucose hexokinase, glucose dehydrogenase pyrroloquinolinequinone (GDH- PQQ), glucose dehydrogenase nicotinamide adenine dinucleotide (GDH-NAD), or glucose oxidase reaction. Ms. Robertson cautioned the audience to know what methodology their instrument uses for testing glucose. She shared a profound example of a patient that was improperly medicated based on an erroneous glucose result:

> "We recently received a report of a patient who suffered irreversible brain damage following an aggressive insulin treatment that was given for elevated glucose readings. Unfortunately, the elevated glucose readings were incorrect because the glucose monitoring device, which was unable to distinguish between glucose and maltose, was reacting to the maltose in the intravenous immunoglobulin solution that the patient was receiving."

She explained that the glucose monitoring system used in this case was the GDH-PQQ method, which cannot distinguish between the sugars glucose, maltose, galactose or xylose. Ms. Robertson also noted that point-of-care glucose testing is a trending tool. The benefits are immediate interaction with the health care provider as well as an increase in patient satisfaction and compliance. She cited the American Diabetes Association recommendation to use point-of-care testing for A1c hemoglobin. This measurement would promote timely decisions on therapy changes when needed.

Vendor Sponsor Board lists the numerous exhibitors and their level of sponsorship from gold to bronze to educational.

The next speaker, Beth Silvers, RD, LDN, CDE, BC-ADM, is a Dietician and Diabetes Educator for Gaston Memorial Home Health Care. Ms. Silvers captivated the audience on the topic, *Teaching What They Do Not Want to Learn*. She instructed participants on how to assess the patient's knowledge of diabetes and his/her willingness to change lifestyle behaviors. Ms. Silvers encouraged everyone to listen actively as they assessed the patient's knowledge and readiness to change. She provided examples of open-ended questions to ask patients before presenting information about the disease. Ms. Silvers also dis-

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cussed the risk factors for complications from diabetes and how to address them with the patient. After the basics of diabetes are shared with the patient, Ms. Silvers noted that the patient should be allowed to set his/her own goals and to take the education process slowly. She also covered the health care behaviors recommended by the American Association of Diabetes Educators: healthy eating, being active, monitoring, taking medication, problem solving, healthy coping, and reducing risks.

The final speaker, Joseph Konen, MD, MSPH, is a Medical and Research Specialist with Pfizer, Inc. and the Chair of the North Carolina Diabetes Advisory Council. Dr. Konen rounded out the conference with his presentation *Diabetes: What is it and What to do about it*. Dr. Konen articulated the seriousness of diabetes in North Carolina. He stated that approximately 8,000 North Carolinians die each year as a result of diabetes and the annual cost of the disease exceeds $1.7 billion in hospitalization charges. He made a profound statement when he noted that obesity is the first chronic disease whose spread looks like an infectious disease epidemic. Dr. Konen stated that obesity is often linked to type 2 diabetes and that the co-existing conditions have led to the coining of a new word, "diabesity®", by the medical community. He explained the metabolic defects in diabetes, an increase in insulin resistance and progressive β cell failure, that contribute to hyperglycemia. Dr. Konen also described the current American Diabetes Association criteria for diabetes and impaired glucose tolerance. He differentiated between the two major types of diabetes, type 1 and type 2, and reviewed the recommendations for disease management and current and future therapies.

The conference also featured vendor exhibits and numerous door prizes, including pedometers and the book *Diabesity: A Doctor and Her Patients on the Front Lines of the Obesity-Diabetes Epidemic*, by Dr. Francine Kaufman, an internationally known authority on obesity and diabetes. Event participants also had access to information, product demonstrations and free samples from the eighteen vendors that sponsored the educational program, including Hemocue, Inc.; Bayer Healthcare; Caligor; Infolab, Inc.; Johnston Therapeutic Wound Healing Center; Kawasumi Laboratories America, Inc.; Laboratory Supply Company; LifeScan, Inc.; Medical Automation Systems; Novo Nordisk Pharmaceutical Industries, Inc.; Smith's Addressing Machine Service, Inc.; and Vashaw Scientific, Inc. Educational exhibitors included NCDPCB, NCSLPH, North Carolina Area Health Education Centers, North Carolina Institute for Public Health, North Carolina Society of Clinical Laboratory Science, and Wake Technical Community College.

As the primary organizer of the conference, the NCSLPH's goal was to offer an educational opportunity that would engage health professionals and promote a better understanding of diabetes and its impact on the health of adults and children. Participants were challenged to use the information they gained to improve the health and quality of life of people with diabetes and to advance the awareness of the disease in their community.

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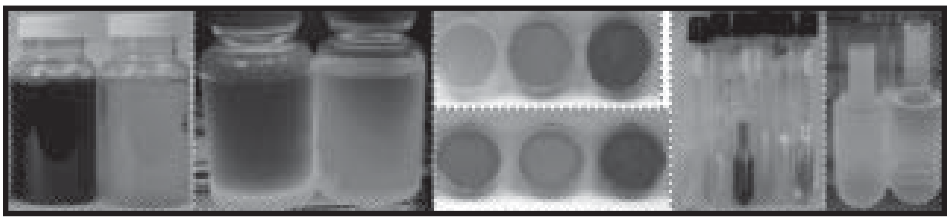
Microbial testing of drinking water



The potential for the spread of disease through water was recognized as early as the 19th century with the implementation of the Interstate Quarantine Act in 1883, which gave the Federal government authority over the quality of drinking water. The first microbiological standards were established in 1912 by the U.S. Public Health Service (US PHS) through the Interstate Carrier Program. These initial standards were revised in 1925, 1942 and 1962, incorporating statistical aspects of testing, lowering the acceptance limits, approving additional analytical techniques, and adjusting the number and location of sampling sites.

Today and throughout history, members of the Coliform group—identified as the genera *Escherichia, Klebsiella, Citrobacter* and *Enterobacter*—have been recognized as potential indicators of the presence of bacterial pathogens in a drinking water supply. These organisms are found in large numbers in the microbial flora of warm-blooded animals, with the number of organisms increasing proportionally in a water supply as the population of surrounding animals increase. Although historically the Coliform bacteria group have not been recognized as indicators of pathogenic protozoa and viruses, the EPA is currently re-evaluating the association of the fecal bacteria *E. coli* with the protozoa *Cryptosporidium*, and is therefore requiring testing for both as part of the Long-Term 2 (LT2) Surface Water Treatment Rule.

Although technological advances in microbial testing have improved the speed at which many pathogens can be detected, the coliform bacteria group is still the best indicator of the potential presence of disease-causing organisms in a water supply. The variety of pathogens which can be present in a drinking water sample is large, but the number of each is often low. However, many of these can be highly infective, even at a low dose. Coliforms are applicable to all water and are usually present when pathogens are, but have little after growth and subsequently are absent when pathogens are not there. These organisms have constant characteristics that make their detection easier.

However, the greatest advantage of the coliform group is the speed and ease with which testing can be performed. The tried and true methods of multiple tube fermentation and membrane filtration are still accepted and used by many laboratories, but these are laborious, time-consuming, and require the purchase and preparation of pre-enrichment and selective growth media. Multiple variations of enzymatic techniques now available can detect Total Coliform and *E. coli* in as little as 18 - 24 hours and are easily read through a colorimetric or fluorescence response. This new technology is now widely used in many EPA compliance-monitoring programs.

The EPA has a number of regulations governing various aspects of drinking water testing. All public water systems must test distribution water samples for the presence/absence of Total Coliform and, if positive, determine if fecal coliforms or *E. coli* are present in the sample. Additional testing is required to determine what remedial action, if any, must be performed. Water treatment plants that use a surface water source must also test this water periodically under the LT2 rule discussed above. Additionally, most plants test their source and finished water daily to optimize their treatment techniques. The Groundwater Rule expected for release later this year will require monitoring of this water source for fecal indicator bacteria.

Many health departments are now performing their own testing in support of their Environmental Health Section. This usually consists of testing for the Coliform group using one of the enzymatic techniques. The bench space and equipment requirements for this testing are minimal, the analytical procedures easy to interpret, and the speed at which results are available are advantageous to many of the public health programs monitored at the local health department.

For additional information on the EPA-approved methods and Certification requirements, please contact
919/807-8879.

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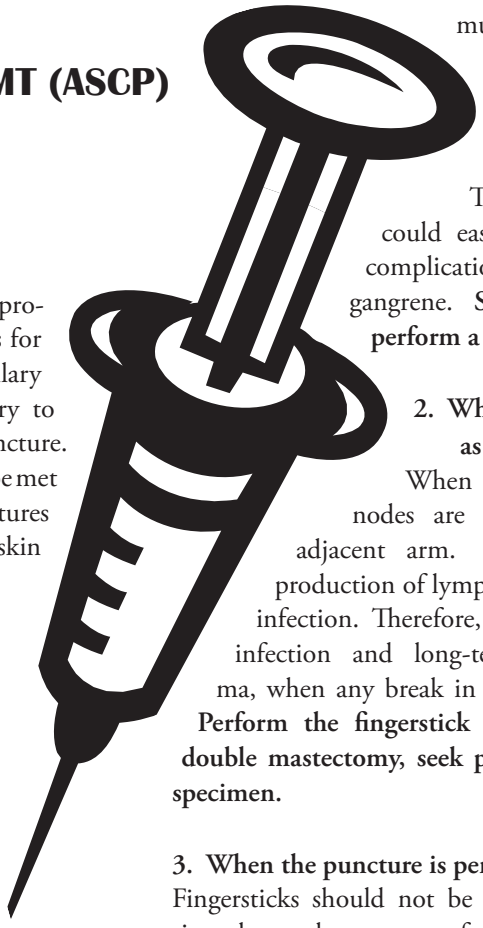
EDITORIAL

Needle Points
By Lisa O. Ballance, BSMT (ASCP)

Phlebotomy Q&A: The Five Pitfalls of Fingerstick Collections



For many patients, skin punctures provide an efficient and convenient means for acquiring a blood specimen. Capillary collections also pose less risk of injury to patients when compared to venipuncture. When laboratory test requirements can be met with small quantities of blood, skin punctures may be especially applicable. Consider skin punctures for the following:

- point-of-care testing;
- patient-performed testing (i.e., diabetic monitoring);
- pediatric patients;
- geriatric patients;
- obese patients;
- severely burned patients;
- patients with thrombotic tendencies; and
- patients in whom superficial veins cannot be successfully accessed.

Capillary blood specimens are typically obtained by performing a fingerstick on older children and adults, and a heelstick on newborns and infants under 12 months of age. However, capillary collections are not appropriate for every patient or test order. More specifically, there are certain situations where a skin puncture to the finger is not acceptable.

So, when is a fingerstick the wrong collection method?

1. When the patient is under 12 months of age.

The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) clearly states in its H4 standard that punctures must never be performed on the fingers of a newborn or infant less than one year old. This is because the distance between the skin and bone in the fingers of these patients is dangerously small, ranging anywhere from 1.2 to 2.2 mm. Therefore, if a fingerstick is attempted, the bone could easily be pierced during the puncture. Potential complications for the infant include local infection and gangrene. **Solution: For infants less than 12 months old, perform a heelstick on a properly prewarmed site.**

2. When the puncture is performed on the same side as a prior mastectomy.

When a patient undergoes a mastectomy, lymph nodes are removed that maintain fluid balance in the adjacent arm. Also diminished by this procedure is the production of lymphocytes, which impacts the body's ability to fight infection. Therefore, mastectomy patients are at increased risk for both infection and long-term, painful swelling, known as lymphedema, when any break in the skin occurs to the affected limb. **Solution: Perform the fingerstick on the non-affected side. In the case of a double mastectomy, seek physician-written approval before collecting the specimen.**

3. When the puncture is performed on an inappropriate site.

Fingersticks should not be performed on previously punctured or swollen sites, due to the presence of excess tissue fluid that may contaminate the blood specimen. When evaluating possible skin puncture sites, the thumb should not be used because it contains a pulse. The index finger should also be avoided since it may be more sensitive or callous than the other fingers. The fifth or "pinky" finger, along with the tips and sides of all fingers, should not be considered because of insufficient tissue depth and the possibility of bone penetration. **Solution: For older children and adults, perform the puncture on the fleshy pad of the middle or fourth finger after ensuring the site is free of trauma and infection.**

4. When test requirements call for a venous specimen.

According to reports cited by CLSI, statistical and/or clinically significant differences in concentration have been observed for some analytes when comparing venous serum with serum/plasma obtained from skin puncture blood. Affected constituents include glucose, potassium, total protein, and calcium. With the exception of glucose, the concentrations of these analytes are lower in skin puncture blood specimens. Although capillary blood specimens are acceptable for routine glucose monitoring of known diabetics, venous specimens are recommended when screening patients for gestational diabetes mellitus (GDM) or performing oral glucose tolerance tests (OGTTs). In addition to diagnostic testing for diabetes, another example where a venous specimen is preferred over a capillary collection is confirmation testing of

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elevated blood lead levels. **Solution: Stay current with and adhere to the testing laboratory's specific specimen collection requirements for all samples you submit for analysis. If in doubt, always verify acceptability of specimen type prior to collection.**

5. When the patient is dehydrated or has poor circulation.
According to CLSI, if a patient is dehydrated or has poor peripheral circulation from other causes (i.e., peripheral edema), it may be impossible to obtain a blood specimen representative of the patient's physiology, especially by skin puncture. **Solution: Consult with the requesting physician. In the case of dehydration, depending on the specific circumstances and the patient's health status, it may be possible to delay collection until the patient has been properly hydrated. However, that's a determination only the requesting provider can make.**

Agencies should ensure those assigned blood collection duties are properly trained, regularly assessed, and have access to all necessary collection procedures and test requirements. In doing so, they arm their staff with critical skills and information that can prevent specimen collection errors and patient injury. Being mindful of the five potential pitfalls associated with fingersticks also goes a long way in guaranteeing the quality and safety of capillary collections. On behalf of the patients you serve, I'll give a "high-five" to that.

North Carolina Public Health Association Laboratory Section News

The North Carolina Public Health Association and North Carolina Association of Local Health Directors will be holding their annual educational conference Oct. 11-13 at the New Bern Convention Center. This year's theme is "Are We Ready?" Information on the conference agenda, conference registration and hotel reservations can be found at NCPHA's website, www.ncpha.com. .

The Laboratory Section will be sponsoring cholesterol and glucose screenings, as well as BMI readings, on Wednesday, Oct. 11, from 1:30 to 5:00 pm and again on Thursday, Oct. 12, from 8:30 to 11:30 am. Special thanks go out to the Onslow County Health Department's Health Watch program and Tammy Horne, Onslow County Health Department Laboratory Supervisor, for co-sponsoring this event. An off-site luncheon is planned (at participant's cost) for Thursday afternoon from 12:15 to 2:15 pm. Anyone interested should contact Debra Springer, Laboratory Section Chair, at (919) 807-8764, so that reservations may be finalized.

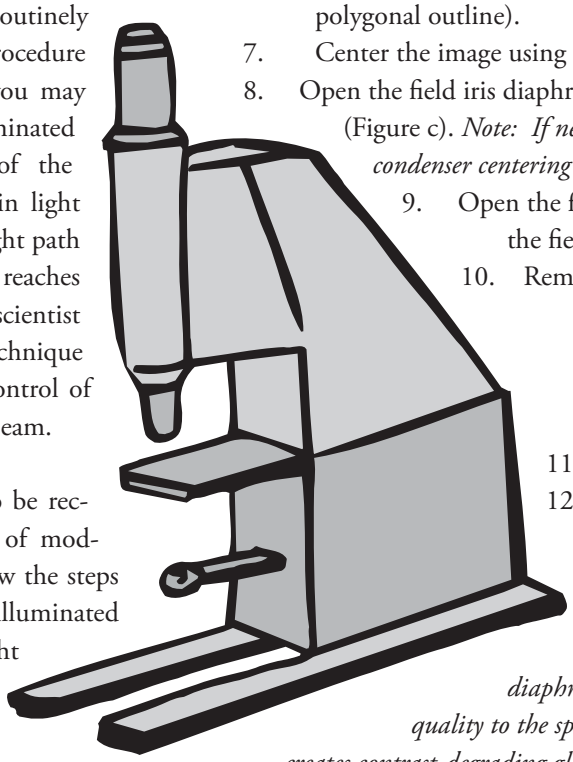
The Laboratory Section will be presenting the Outstanding Laboratorian of the Year award at this year's business meeting. The Laboratory Section will also have a membership incentive drawing for a chance to have NCPHA dues paid for one year. Sponsor a new NCPHA member to be eligible for the drawing.

Please consider joining the Laboratory Section, NCPHA and NCALHD in October in beautiful New Bern, N.C.

Microscope Tips

Koehler Illumination Made Easy

Are you getting the best image possible from your microscope? If you do not routinely perform the Koehler Illumination procedure on your light microscope, then you may be experiencing an unevenly illuminated image, glare, and overheating of the specimen. For optimum results in light microscopy, it is crucial that the light path be set properly before the light reaches the specimen. In 1893, German scientist August Koehler introduced this technique as a method for applying exact control of the light path in the illuminating beam.



Koehler Illumination continues to be recommended by all manufacturers of modern laboratory microscopes. Follow the steps described below to produce an illuminated specimen that is uniformly bright and free from glare. This will allow the user to realize the microscope's full potential.

Equipment Specifications:

The microscope must have a vertically adjustable, centerable condenser and iris diaphragm. A clean microscope will give the best results.

Procedure:

Review basic parts of a microscope (Diagram 1) before beginning.

1. Turn the lamp to the lowest setting.
2. Raise the condenser to the highest setting.
3. Open the aperture iris (in the condenser) and the field iris (in the base) diaphragms all the way.
4. Focus on a specimen with the 10X objective.
5. Close down the lamp field iris diaphragm while viewing. A small circle of light should be visible in the eyepieces (Figure a)
6. Lower the condenser slightly until the diaphragm image is in focus (a polygonal outline).
7. Center the image using the condenser centering screws (Figure b).
8. Open the field iris diaphragm to the edge of the field of view (Figure c). *Note: If needed, re-center the image by adjusting the condenser centering screws.*
9. Open the field iris diaphragm until the image just clears the field of view (Figure d).
10. Remove an eyepiece and check to see that 65-75% of the visible aperture is filled with light. Adjust the aperture iris diaphragm to achieve this level of illumination (Figure e).
11. Replace the eyepiece.
12. Repeat step 10 after switching to a different objective. With experience, you will be able to judge the correct level of illumination without removing an eyepiece. *Note: Closing the aperture diaphragm too far produces a refractile quality to the specimen due to diffraction. Opening it too far creates contrast-degrading glare.*

Once the procedure is complete, nearly optimal illumination of a specimen should be achieved and the field iris diaphragm and condenser height should not be adjusted. To reduce glare and improve contrast, adjust the light intensity or the aperture iris diaphragm and use filters, as needed. **If either the field iris diaphragm or the condenser height is changed after setting the microscope for Koehler Illumination, the procedure must be repeated.**

Remember the old adage "practice makes perfect"? Over time, practicing Koehler Illumination will improve your skill in performing the procedure. However, remember that improper practice often leads to poor results. If you have trouble achieving proper illumination with your microscope, consider taking the Microscopy class offered throughout the year at the N.C. State Laboratory of Public Health. Check on-line for a class schedule at *http://slph.state.nc.us/LabImprovement* or call 919/733-7186.

Submitted by:
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Microscope Tips cont. from page 10

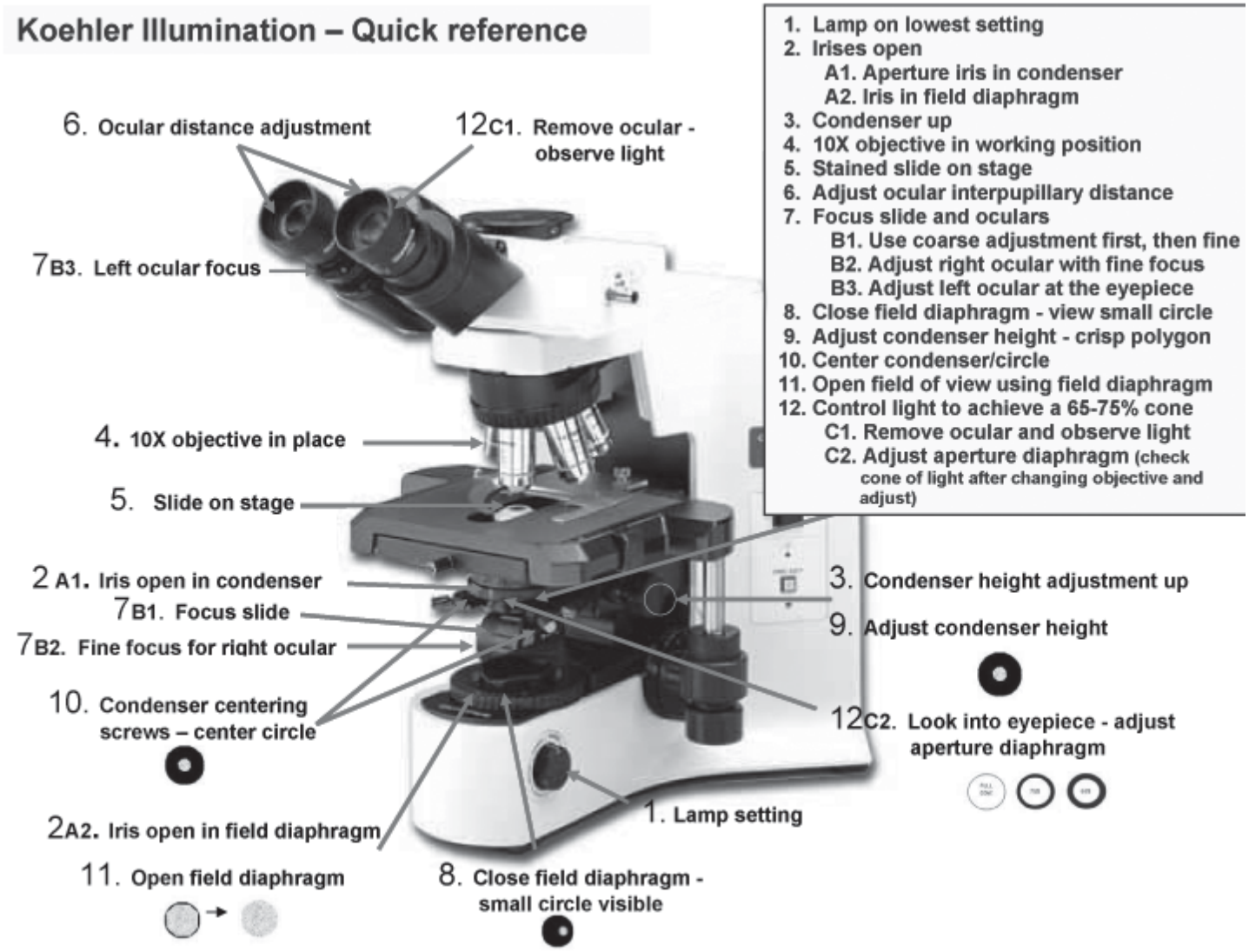
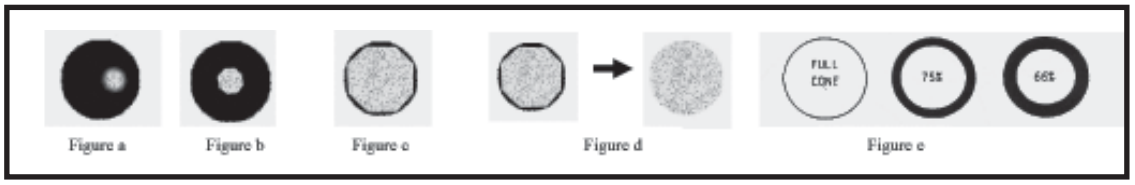


Diagram 1



Lab Test of the Quarter

Cholesterol

Also Known As
Blood Cholesterol, Total Cholesterol

Related Tests
HDL, LDL, Triglycerides, Lipid Profile

Sample Type
Blood (Capillary or Venous), Serum, Plasma

Normal Findings
Adult/Elderly: <200 mg/dl
or 5.20 mmol/L
(SI units)
Child: 120-200 mg/dl
Infant: 70-175 mg/dl
Newborn: 53-135 mg/dl1

What is Cholesterol?
Cholesterol is a steroid that is essential for life. It is involved in the production of other steroids; sex hormones related to growth, development, and reproduction; and bile acids that are needed to absorb nutrients from food. It also forms the membranes of all cells that make up the tissues and organs of the human body. A great deal of the cholesterol we eat comes from eating meat products. It is metabolized in the liver and is transported through the bloodstream in the form of lipoproteins. Twenty-five percent of cholesterol is bound to high-density lipoproteins (HDL), which carry excess cholesterol out of the body for disposal. This is known as "good" cholesterol. Approximately 75 percent of cholesterol is bound to low-density lipoproteins (LDL), which deposit cholesterol in tissues and organs. This is known as "bad" cholesterol.[1, 2] High cholesterol levels can be due to heredity, a high-fat diet, various disease states, or a combination of the above.

Current recommended lipid levels

	European guideline	US guideline
Total cholesterol	less than 5.0 mmol/l	less than 240 mg/dl (6.2 mmol/l)
LDL-cholesterol	less than 3.0 mmol/l	less than 160 mg/dl (3.8 mmol/l)
HDL-cholesterol	1.0 mmol/l or more in males 1.2 mmol/l or more in females	40 mg/dl (1 mmol/l) or more
Triglycerides (fasting)	less than 1.7 mmol/l	less than 200 mg/dl (2.3 mmol/l)

http://www.who.int/cardiovascular_diseases/en/cvd_atlas_06_lipids.pdf

Why Test Cholesterol?
The main reason cholesterol is tested is to identify patients who are at risk for arteriosclerotic heart disease. It is a screening test and is not generally used to diagnose or monitor a disease, but rather is used to calculate risk. High cholesterol causes a hardening of the arteries and heart disease, which lead to an increased risk of heart attacks. This screening test, usually performed in the form of a lipid panel (Total Cholesterol, HDL, LDL, and Triglycerides), should be done on adults over age 20 at least every five years as part of their regular preventive care. Patients who are taking medications or modifying their diet to decrease their cholesterol levels may be tested more frequently to assess their progress.

Total Cholesterol as an Indicator of Risk of Coronary Heart Disease

Age	Low Risk	Moderate Risk	High Risk
2-19	<170	171-185	>185
20-29	<200	201-220	>220
30-39	<220	221-240	>240
>40	<240	241-260	>260

Interfering Factors
It is standard protocol to measure cholesterol readings after a person has fasted for 12 to 14 hours.[1] Other sources state that cholesterol levels are not affected by a single meal and fasting is not necessary.2 It should be noted, however, that diet will affect cholesterol levels at least two weeks prior to the test, and levels will change within several weeks of changing from a high-fat to a low-fat diet.[1,2] It should also be noted that patients should be healthy, as acute illness can affect test results. Factors that falsely elevate cholesterol include pregnancy, oophorectomy, and certain drugs such as adrenocorticotropic hormone, anabolic steroids, beta-adrenegic blocking agents, corticosteroids, epinephrine, oral contraceptives, phenytoin (Dilantin), sulfonamides, thiazide diuretics, cyclosporine, and vitamin D. Factors that falsely decrease cholesterol

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Lab Test cont. from page 12

include a recumbent position and drugs that include allopurinol, androgens, bile salt-binding agents, aptopril, cholopropamide, clofibrate, colchicines, colestipol, erythromycin, isoniazid, liothyronine (Cytomel), lovastatin (Mevacor), monoamine oxidase inhibitors, neomycin (oral), niacin, and nitrates.[1]

Abnormal Findings

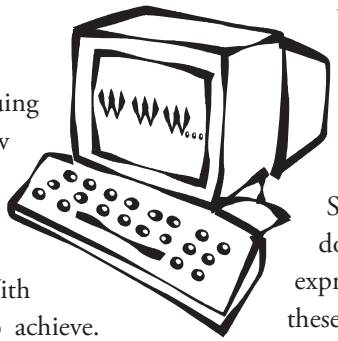
There are many different reasons why a person may have an abnormal cholesterol measurement.

Increased Levels		Decreased Levels
Hypercholesterolemia	Atherosclerosis	Malabsorption
Hyperlipidemia	Biliary cirrhosis	Malnutrition
Hypothyroidism	Stress	Hyperthyroidism
Uncontrolled diabetes mellitus	Nephrosis[1]	Cholesterol-lowering medication
Nephrotic syndrome		Pernicious anemia
Pregnancy		Hemolytic anemia
High-cholesterol diet		Sepsis
Xanthomatosis		Stress
Hypertension		Liver disease
Myocardial infarction		Acute myocardial infarction

References
1. Pagana KD, Pagana TJ. Mosby's Diagnostic and Laboratory Test Reference.
4th ed. St. Louis, Mo: Mosby, 1999.
2. Cholesterol. Lab Tests Online. 2004. Available at http://www.labtestsonline.org/understanding/analytes/cholesterol/glance.html.
Accessed August 7, 2006.

Submitted by:
Jennifer A. Anderson, BS, MT(ASCP)CM
Laboratory Improvement Consultant

Learning On-Line



Due to rapid advances in medical technology, continuing education is vital to laboratorians. It is important to review information previously learned to maintain current skills, and to continuously learn new information in order to provide the best possible care for patients. Often, employers require a certain number of hours of continuing education each year as well. With budgetary and staffing constraints, this is sometimes difficult to achieve. Fortunately, there are many sources of laboratory continuing education, many of them FREE or very inexpensive, on the Internet. We will be sharing some of our favorites each quarter in the Lab-Oratory. Three such sites are

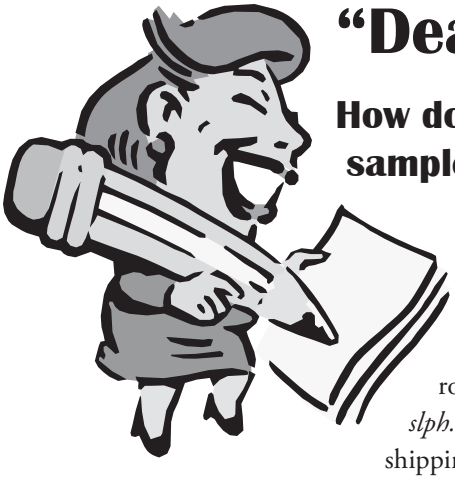
http://dnamededcafe.com/home.php

http://laboratorian.advanceweb.com/common/learningscope/learningscope.aspx

http://www.gcflearnfree.org/

We have provided the links to these sites because they have information that may be of interest to our readers. The State of North Carolina and the N.C. State Laboratory of Public Health do not necessarily endorse the views expressed or the facts presented on these sites. Further, the State of North Carolina and the N.C. State Laboratory of Public Health do not endorse any commercial products or information that may be presented on or could be advertised on these sites.

Submitted by
Jennifer Anderson, BS, MT(ASCP)CM
Consultant, Laboratory Improvement



"Dear Lab-bey"

How does one submit a suspected Select Agent or suspect sample to the NCSLPH for identification or rule-out?

According to Royden Saah, Bioterrorism and Emerging Pathogens (BTEP) Coordinator, the proper procedure is as follows. First, **call the BTEP section PRIOR to submission of ANY isolate or sample**. Phone 919-807-8765 (M-F, 8-5 PM) or 919-807-8600 (after-hours/holiday/emergency). Next, complete the Bioterrorism submission forms (Environmental or Clinical, DHHS T806 or 3431) located on the NCSLPH homepage *http://slph.state.nc.us*. Ship the sample or isolate in a proper containment system, following all IATA shipping regulations, or hand-deliver it as soon as possible. Clearly label the exterior packaging "sample for BTEP". Environmental samples MUST be submitted by a law enforcement agency or PHRST team. Environmental samples are normally delivered by a law enforcement representative and are accompanied by a chain-of-custody document.

"Dear Lab-bey..."

If you have a technical laboratory question that you would like to have answered please submit it to: Jennifer.A.Anderson@ncmail.net. The answer to your question may be featured in the next edition of Lab-Oratory.

The Safety Corner

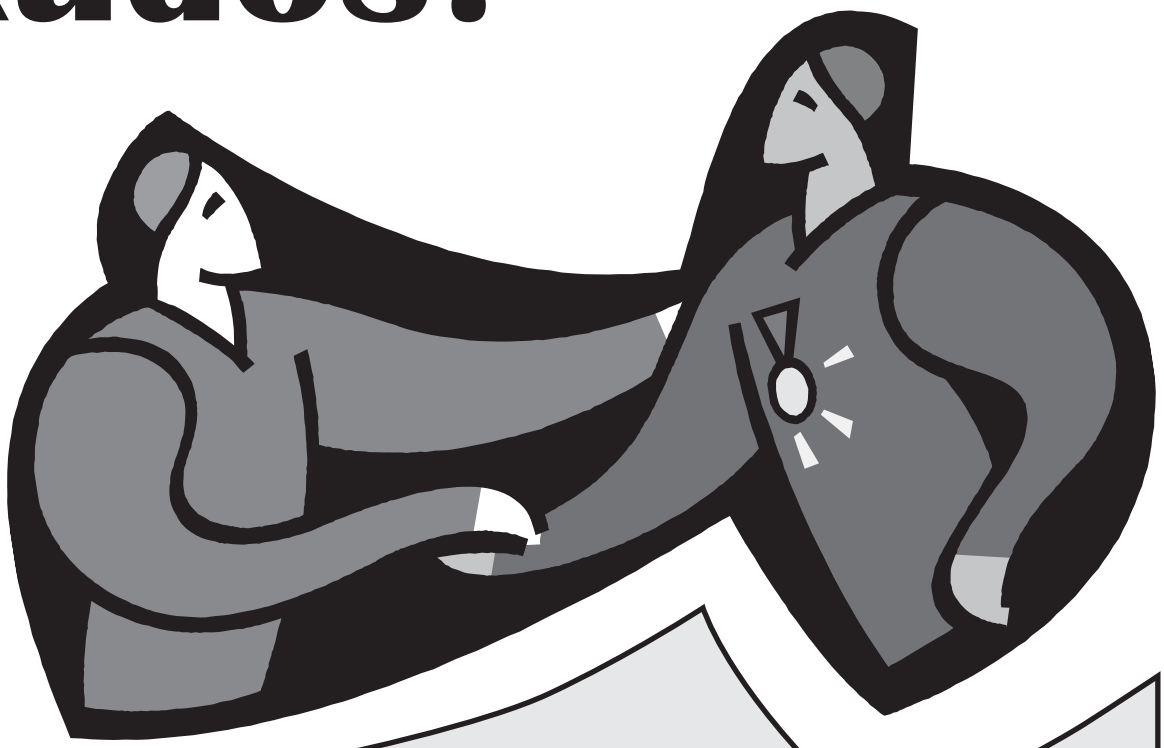
Exposure Control Plan Series-Employee Education and Training

OSHA's Bloodborne Pathogens Regulation states that all employees with potential for occupational exposure must participate in a training program. This training must be provided during work hours and at no cost to the employee. Training should be offered at time of initial employment and annually thereafter. Certain topics are covered in this annual training, including:

- OSHA's Bloodborne Pathogen Standard
- Overview of bloodborne pathogens and modes of transmission
- The Exposure Control Plan
- Work practice controls
- The use and limitations of engineering controls
- Personal protective equipment: selection, use, removal, and disposal
- Hepatitis B vaccine
- Steps to take in an emergency involving blood or other potentially infectious materials
- Post-exposure reporting and follow-up

Cont. on page 19

Kudos!



In spring 2005, the NCSLPH began naming a State Lab Employee of the Month. Employees are encouraged to nominate co-workers who demonstrate great work ethics and always lend a helping hand. In June, Donna Goodmond in Administration was honored; Germaine Buchanan of Administration was the July recipient of the award. The August recipient was Tony Ivosic from our Quality Assurance branch. Congratulations to all of our winners and thank you for your contributions to the NCSLPH!

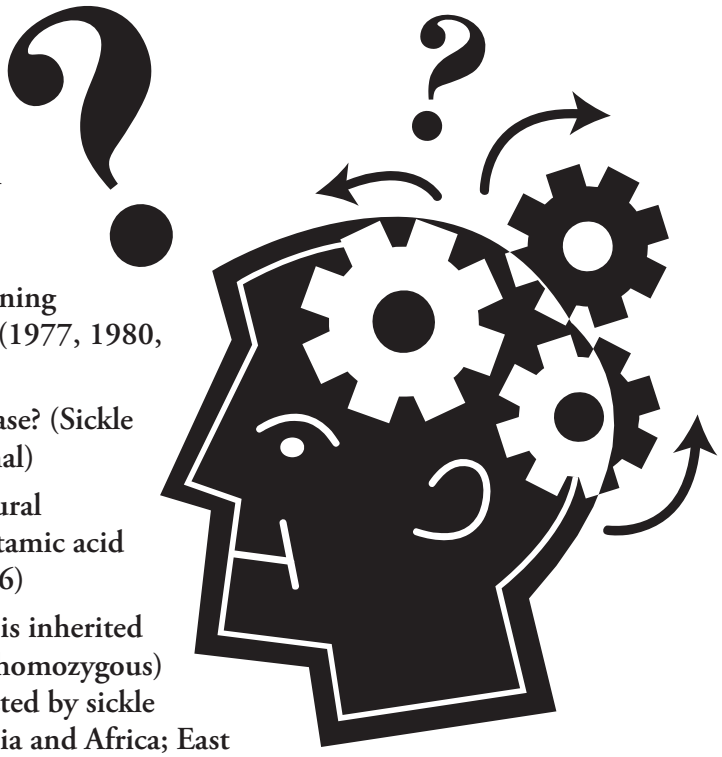
Sharon Robertson, Craven County Health Department, was awarded the Laboratorian of the Year for EDNCPHA Laboratory Section of 2006. Congratulations Sharon!

Gail Peterson, Mitchell County Health Department, received the 2006 WNCPHA Beth Fletcher Laboratorian of the Year Award on May 24. Congratulations Gail!

Robeson County Health Department just recently passed their COLA inspection with only one citation! Also recently, they passed their accreditation. Great job to the lab staff and to all of the staff at Robeson County Health Department!

Please contact Kristy O'Briant at (919) 733-7186 or kristy.obriant@ncmail.net if you would like to recognize a co-worker at your facility.

Brain Exercise



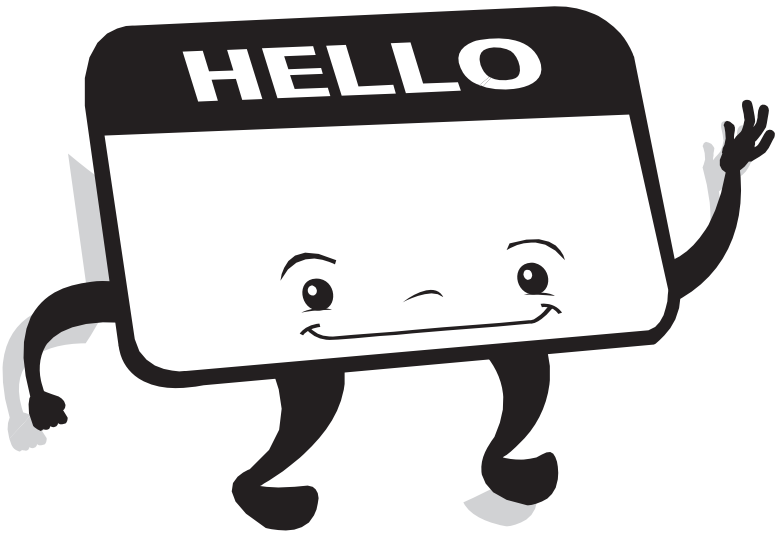
Test your laboratory knowledge. Select the best answer(s).

1. What is the most common abnormal hemoglobin detected by newborn screening programs? (Hb S, Hb C, Hb A, Hb E)
2. When did North Carolina initiate statewide screening for abnormal hemoglobins in non-white infants? (1977, 1980, 1987, 1990)
3. What is the most common type of sickle cell disease? (Sickle cell anemia or Hb SS, Hb SC, Hb S/D, Hb S-βthal)
4. What is the amino acid substitution in the structural formula for Hb S (α2β26 valine) ? (valine for glutamic acid at position 6, glutamic acid for valine at position 6)
5. Sickle cell trait is a benign condition where Hb S is inherited with Hb A in what type of state? (heterozygous, homozygous)
6. What is the primary ancestry of populations affected by sickle cell anemia? (Africa, India and Mediterranean; Asia and Africa; East Europe and Asia)
7. What are the symptoms of sickle cell anemia? (extreme swelling of extremities and pain, hemolytic anemia, chronic organ damage, infection)
8. What is the estimated incidence of sickle cell anemia in live African-American births? (1 in 1000, 1 in 375, 1 in 1500)
9. What is the estimated occurrence of sickle cell trait in African Americans? (20%, 3%, 8%, 15%)
10. What happens to the red blood cells in a person with Hb S when the blood is deoxygenated? (cells retain their normal biconcave shape; cells become less soluble, rigid and sickle shaped)
11. In what year did North Carolina add screening for sickle cell anemia to the newborn screening panel as a universal test for all infants, regardless of ethnicity? (1990, 1994, 1995, 1999)
12. Abnormal or inconclusive hemoglobin results on the newborn screen initiate what action? (NCSLPH requests additional testing on whole blood from the infant and biological parents, additional testing of the newborn is submitted on the filter paper form to the NCSLPH)
13. Infants with sickle cell disease are characteristically without symptoms until the second half of the first year of life due to the protective effect of what hemoglobin? (Hb C, Hb A, Hb F, Hb D)
14. What is the method the NCSLPH uses to screen for abnormal hemoglobins? (isoelectric focusing, high performance liquid chromatography, sickledex)
15. What confirmatory test for abnormal hemoglobins was added in 1997 at the NCSLPH? (isoelectric focusing, high performance liquid chromatography, sickledex)

Questions submitted by:
Colleen Miller, BS MT(ASCP)
Laboratory Improvement Consultant

Who's New in Public Health?

The following are this quarter's newcomers to North Carolina's Public Health arena. We would like to extend a warm welcome to you all. As always, we hope you will continue to stay with us and will find your job both enjoyable and fulfilling as you serve the citizens of North Carolina.



The North Carolina State Lab of Public Health would like to welcome the following:

Derrick White in Scientific Services, Erik Hrebenuyuk in the CytoPrep Lab, Norman Good in the Environmental Organic Chemistry Unit, and Shawna Carrigan and Theresa Obong in FIA/GAL/BIO.

The following are departures from the Toe River Health District: Elaine Dellinger and Becky Petrich have both left Yancey County. Gail Peterson left Mitchell County August 18. We wish these ladies the best of luck in their future endeavors.

If you would like to recognize new employees in your facility, please email their information to crystal.poppler@ncmail.net.

Brain Exercise Answers:

1. Hb S; 2. 1987; 3. Sickle cell anemia or Hb SS; 4. valine for glutamic acid at position 6; 5.heterozygous; 6. Africa, India and Mediterranean; 7. extreme swelling of extremities and pain, hemolytic anemia, chronic organ damage, infection; 8. 1 in 375; 9. 8%; 10. cells become less soluble, rigid and sickle shaped; 11. 1994; 12. NCSLPH requests additional testing on whole blood from the infant and biological parents; 13. Hb F; 14. isoelectric focusing; 15. high performance liquid chromatography

Virology/Serology

I J S G S R A O A D L E S S B D H R W S Y S S G F
M M L I Q U J R G L V P E D M J Y O E I D I I A F
E C M I T E R B B I L R I E L A H C S X T T T C S
Q H X U N I W I T O O E B T S X E K T A S I I C I
B U L P N W L C V D V Y B S K Q P Y N L U L L Y G
D U T I N O A A I S X I A U X L A M I Y R A A S A
V K A U O E G A H A E O R U R T T O L H T H H B R
T I J D R M G L F P N I M U N G I U E P I P P C W
X Q U N M N G F O U E K B X S O T N V O Y E E R X
F B O E O X X F M B L C L A T N I T I R G C C C W
E N K S M E A M Q R U J N Q R P S A R P Q N N D L
I I I S D Z I J T O U L F E H E I I U P J E E K J
P S I X Z E B H H O S K I B E P A N S L J S E G N
X E L P M I S S E P R E H N Z S Z S F N L I N J T
W P N Y T I N U M M I W G R P R S P Q G F U I M W
W L Z M U M U G I O S E Z Z V C V O M R G O U P X
Y N D B Q V M E Z S G X W E T A F T R T D L Q B Q
E V L Z M L G D X W Y D S H R N I T R C V T E R P
T R E P O N E M A P A L L I D U M E C H A S N P I
N M X W T N R Z F W X W C H U B A D G A D L R Q E
S I T I L A H P E C N E A I N R O F I L A C E X P
H I I X Q M K E O X L B Y L D Z J E T O X R T A N
Y S Q O K Y L A R L L E N N J H K V I S D O S B Z
D K B L N E I O A B I V M E L J E E F G N I A C S
T W E V X R I C K E T T S I A O E R B W W P E P R

ARBOVIRUS
CALIFORNIA ENCEPHALITIS
EASTERN EQUINE ENCEPHALITIS
ENZYME IMMUNOASSAY
HEPATITIS
HERPES SIMPLEX
IMMUNITY
IMMUNOGLOBULIN
LACROSSE ENCEPHALITIS
NONREACTIVE
PROPHYLAXIS
RABIES VIRUS
RICKETTSIA
ROCKY MOUNTAIN SPOTTED FEVER
RUBELLA
SERODIAGNOSIS
ST LOUIS ENCEPHALITIS
TREPONEMA PALLIDUM
TRUST
VARICELLA
WEST NILE VIRUS

Created by Puzzlemaker at DiscoverySchool.com

The Safety Corner cont. from page 14

- The use of signs and labels in the laboratory

Although the information in this training is extremely important, it does not have to be boring. Try ways to make this training enjoyable with different games and prizes. Possibly categorize safety questions into a Jeopardy format and reward the winning team with candy. Get the students involved by having them perform demonstrations for the other participants. With a little creativity, training is not painful at all!

Look for the next installment of the Exposure Control Plan series in the next Lab-Oratory when control measures will be discussed!

Article submitted by
Kristy O'Briant, BS, Laboratory Improvement Consultant, NCSLPH

UPCOMING EVENTS . . .

September 29, 2006	**State Lab Orientation***
October 11, 2006	**Advanced Microscopy: Viewing and Reviewing ***
October 12, 2006	**Wet Mount Workshop***
October 25, 2006	**Basic Microbiology and Gram Staining Workshop (Cancelled—look for this workshop in our 2007 Training Bulletin)***
November 1-2, 2006	**Lab Methods in the Diagnosis of Gonorrhea***
November 8, 2006	**Policy and Procedure Writing***
November 14-17, 2006	**Bacteriologic Methods in the Analysis of Drinking Water***
November 30, 2006	**Bioterrorism Workshop***

**Additional information for laboratory improvement workshops and applications can be found on the SLPH web site at http://slph.state.nc.us/LabImprovement/default.asp.*

The Oct. 25 Basic Microbiology and Gram Staining Workshop has been cancelled—look for this workshop in our 2007 Training Bulletin.**

*****Please look for our 2007 Training Bulletin, due out in November!***

Laboratory Improvement
P.O. Box 28047
Raleigh, NC 27611

Lab-Oratory can also be found on the web at http://slph.state.nc.us/ under "Lab Improvement".

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